HABC Gentoype Data Submission Form

Submitting Investigator	
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Lab Contact	
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Date Submitted	3/8/05
Polymorphism Information	
Name	Beta-2 adrenergic receptor
RS# or Unique Identifier	rs1042713, rs1042714
Gene	ADRB2
Chromosome position	5q31-33
Alleles	rs1042713: A/G; rs1042714: C/G
Assay Information	
Genotyping Method	PCR amplification of allele specific primers (PASA)
Genotypes in HWE (Y/N) (attach HWE Form)	Y
Amount of DNA used	10 ng
PCR primers	For rS1042713: FW- CTTCTTGCTGGCACCCA**T**TA FM- CTTCTTGCTGGCACCCA**T**TG R- CAGGCCAGTGAAGTGATGAA
	For rS1042714: FW- GGACCACGACGTCACGCA**A**C FM- GGACCACGACGTCACGCA**A**G R- TGATGAAGTAGTTGGTGACC
PCR Components **and** Concentrations (Taq, buffer, $MgCl_2$, primers, DMSO, other reagents)	Each reaction mixture contained a 1:12,500 dilution SYBR Green I nucleic acid gel stain 10000X in dimethyl sulfoxide (DMSO) (Molecular Probes, Eugene, OR), 0.2 mM dNTP mixture, 200 nM of both forward and reverse primer, 1U Taq DNA Polymerase (Promega, Madison, WI), 6% DMSO, 1X SmartCycler additive reagent (a 5X additive reagent containing BSA at 1 mg/mL, Trehalose at 750 nM, and Tween-20 at 1% v/v) (Cepheid, Sunnyvale, CA), and 10 ng genomic DNA in 1X PCR Buffer (pH 8.3, 10X solution containing 100 mM Tris HCl, 500 mM KCl, and 15 nM $MgCl_2$ and 0.01% Gelatin) (Sigma, St. Louis, MO).
PCR Cycling conditions (time and temperature for each step, # of cycles, etc.)	The amplification program consisted of initial denaturation of 95°C (5 min) followed by 27 cycles of 95°C (15 s), annealing at 60°C (45 s), and extension at 72°C (30 s). After amplification, melt analysis was performed by heating the reaction mixture from 60°C to 95°C at a rate of 0.2°C/s.
Detection Oligo(s) (if applicable)	PCR products containing both the sites of polymorphism at positions 16 and 27 were generated for sequencing using the Sense primer : 5'-GCTCACCTGCCAGACTGC-3'
	Antisense primer: 5'-CAGCAGGTCTCATTGGCATA-3'
Other Reaction Conditions (detection reaction components, incubation conditions, gel %, etc.)	The genotyping method was validated using direct sequencing (ABI Prism® 3100, Applied Biosystems, Foster City, CA) after the PCR products were isolated by QIAquick (Qiagen, Valencia, CA). Amplification reactions were also routinely checked for the presence of nonspecific products by 1% agarose gel electrophoresis.
Other Assay Info	Additional info on the assay can be obtained from *Clin Chim Acta* 341(1-2):93-100.